QUICK GUIDE

Agarose Gel Electrophoresis

WHAT IS ELECTROPHORESIS?

Electrophoresis is a technique that allows us to separate DNA, RNA or proteins according to their size.

WHAT DO I NEED TO SEPARATE A MIXTURE OF DNA MOLECULES?

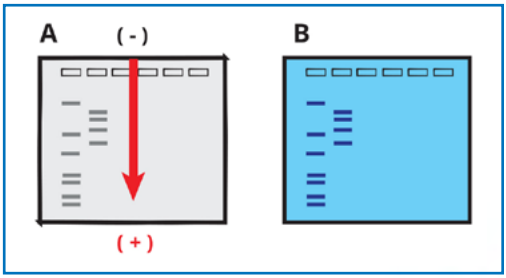
In addition to your DNA sample, you will need:

- GEL LOADING SOLUTION – includes glycerol to help DNA samples enter into the wells and a visible dye to monitor migration through the gel.
- AGAROSE – a polysaccharide used as the separation matrix.
- ELECTROPHORESIS BUFFER – contains ions necessary to conduct an electrical current, maintains pH of experiment.
- HORIZONTAL ELECTROPHORESIS APPARATUS – holds the buffer and the gel, has positive and negative electrodes.
- POWER SUPPLY – generates the current necessary to move DNA through gel.
- MICROPIPETTE – used to transfer samples into wells.
- A special STAIN that allows us to visualize DNA.

HOW DOES ELECTROPHORESIS SEPARATE DNA FRAGMENTS?

The mixture of DNA molecules is added into depressions (or "wells") within a gel, and then an electrical current is passed through the gel. Because the sugar-phosphate backbone of DNA has a strong negative charge, the current drives the DNA through the gel towards the positive electrode (Figure A).

At first glance, an agarose gel appears to be a solid at room temperature. On the molecular level, the gel contains small channels through which the DNA can pass. Small DNA fragments move through these holes easily, but large DNA fragments have a more difficult time squeezing through the tunnels. Because molecules with dissimilar sizes travel at different speeds, they become separated and form discrete "bands" within the gel. After the current is stopped, the bands can be visualized using a stain that sticks to DNA (Figure B).



TIMSTAR

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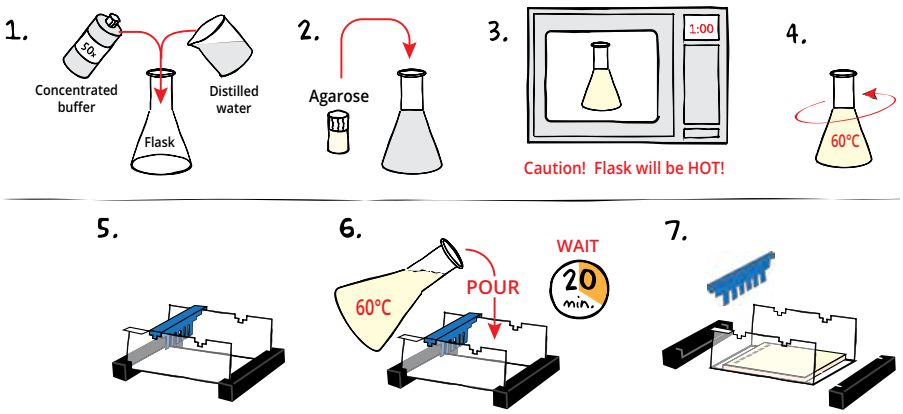
EDVOTEK®

Casting the Agarose Gel



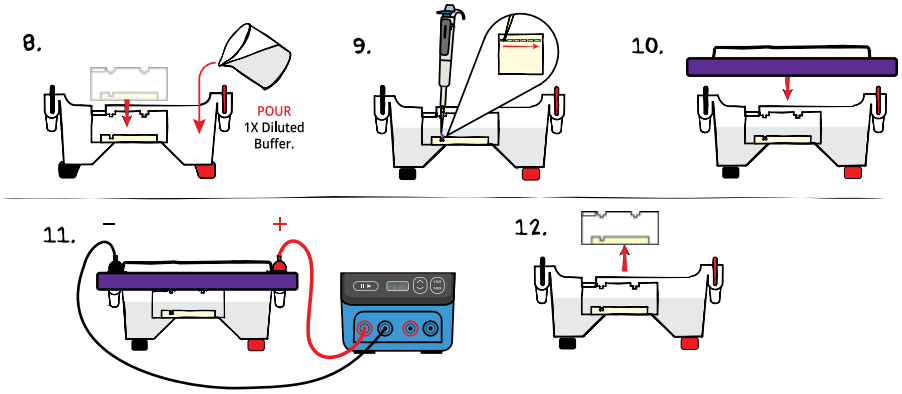

Wear gloves and safety goggles

1. **DILUTE** concentrated 50X Electrophoresis buffer with distilled water (refer to Table A for correct volumes depending on the size of your gel casting tray).
2. **MIX** agarose powder with buffer solution in a 250 mL flask (refer to Table A).
3. **DISSOLVE** agarose powder by boiling the solution. **MICROWAVE** the solution on high for 1 minute. Carefully **REMOVE** the flask from the microwave and **MIX** by swirling the flask. Continue to **HEAT** the solution in 15-second bursts until the agarose is completely dissolved (the solution should be clear like water).
4. **COOL** agarose to 60 °C with careful swirling to promote even dissipation of heat.
5. While agarose is cooling, **SEAL** the ends of the gel-casting tray with the rubber end caps. **PLACE** the well template (comb) in the appropriate notch.
6. **POUR** the cooled agarose solution into the prepared gel-casting tray. The gel should thoroughly solidify within 20 minutes. The gel will stiffen and become less transparent as it solidifies.
7. **REMOVE** end caps and comb. Take particular care when removing the comb to prevent damage to the wells.

Table A — **Individual 0.8% UltraSpec-Agarose™ Gels**

Size of Gel Casting tray	Concentrated Buffer (50x)	+ Distilled Water	+ Amt of Agarose	= TOTAL Volume
7 x 7 cm	0.6 mL	29.4 mL	0.24 g	30 mL
10 x 7 cm	0.9 mL	44.1 mL	0.36 g	45 mL
14 x 7 cm	1.2 mL	58.8 mL	0.48 g	60 mL

Running the Gel



8. **PLACE** the gel (still on the tray*) into the electrophoresis chamber. COVER the gel with 1X Electrophoresis Buffer (See Table B for recommended volumes). The gel should be completely submerged.
9. **LOAD** the samples into the wells in the order indicated by your instructor.
10. **PLACE** safety cover on the unit. **CHECK** that the gel is properly oriented. Remember, the DNA samples will migrate toward the positive (red) electrode.
11. **CONNECT** leads to the power source and **PERFORM** electrophoresis (See Table C for time and voltage guidelines). Allow the tracking dye to migrate at least 3 cm from the wells.
12. After electrophoresis is complete, **REMOVE** the gel and casting tray from the electrophoresis chamber.

PROCEED to staining and visualizing agarose gels using FlashBlue™ Stain.

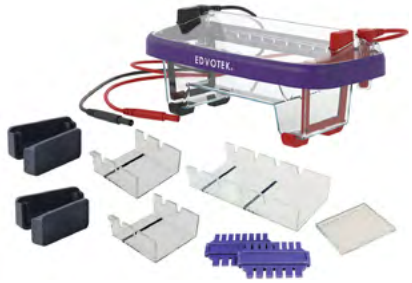
Table B — **1x Electrophoresis Buffer (Chamber Buffer)**

Timstar Model #	Total Volume Required	Dilution: 50x Conc. Buffer	+ Distilled Water
BT180800 (M12)	400 mL	8 mL	392 mL
BT97820 (M36)	1000 mL	20 mL	980 mL

Table C — **Time and Voltage** (0.8% Agarose Gel)

Model	BT180800 (M12) or BT97820 (M36)
Volts	Min/Max (minutes)
150	20/35
125	30/45
100	40/60

Related Products



M12 Complete Electrophoresis Package
Code: BT180800

M36 HexaGel Electrophoresis Apparatus
Code: BT97820

Agarose Powder
20 grams
Code: BT100518

100 grams
Code: BT100520

Electrophoresis Buffers
50x TAE, 100 mL
Code: BT100530

50x TAE, 500 mL
Code: BT140585

10x TBE, for 5 L
Code: BT110100

Gel Loading Solution
10x Yields 6 mL final volume of DNA sample.
Code: BT140582

FlashBlue DNA Stain
10x Concentrate for 1.2 L
Code: BT150616

DuoSource Power Supply
75 or 150 V
Code: BT150802

Fixed Volume Minipipettes

5 µL	*Code: BT97835*
10 µL	*Code: BT97837*
20 µL	*Code: BT97839*
40 µL	*Code: BT97843*
50 µL	*Code: BT97845*
100 µL	*Code: BT97847*

Details for all these products and MORE can be found on our website!